Synthesis of distamycin A polyamides targeting G-quadruplex DNA

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A number of amide-linked oligopyrroles based on distamycin molecules have been synthesized by solid-state methods, and their interactions with a human intramolecular G-quadruplex have been measured by a melting procedure. Several of these molecules show an enhanced ratio of quadruplex *vs.* duplex DNA binding compared to distamycin itself, including one with a 2,5-disubstituted pyrrole group. Quadruplex affinity increases with the number of pyrrole groups, and it is suggested that this is consistent with a mixed groove/G-quartet stacking binding mode.

Introduction

The telomerase enzyme complex, which catalyses the synthesis of telomeric DNA repeats, is responsible for the maintenance of telomere integrity in cancer cells, and plays a major role in their immortalisation.[1–3] Telomerase, which is expressed in 80–90% of cancer cells, and not significantly up-regulated in normal somatic cells, is therefore a key target for selective therapeutic intervention.[4] One particular strategy involves the targeting not of the enzyme *per se*, but its substrate, the single-stranded 3′ overhang of telomeric DNA.[5] In this approach, small-molecule telomere targeting agents induce this guanine-rich DNA to fold into an intramolecular quadruplex structure, which cannot be recognized by the RNA template domain of telomerase and so cannot act as a substrate for the enzyme itself.[6] In addition quadruplex formation may dissociate telomere ends from physical association with telomerase and other telomere-binding proteins,[7] which in cells then results in the triggering of a DNA damage response and eventual cell death.[8] A large number of such small quadruplex-binding molecules have been studied,[9–16] the majority of which have high affinity for quadruplex DNAs by virtue of their possessing a planar aromatic chromophore such as an acridine, anthraquinone or porphyrin, which can interact with the planar G-quartet surface of a quadruplex by π–π interactions. A lead compound, BRACO-19, from one such series, of trisubstituted acridines, has selectivity for quadruplex *vs.* duplex DNA,[14] and shows cellular effects consistent with G-quadruplex formation and telomere targeting[17] as well as demonstrating *in vivo* antitumour activity in a xenograft model.[18]

An extended heteroaromatic chromophore is not an essential feature of quadruplex-binding ligands, as shown by molecules such as the cyclic oxazole natural product telomestatin,[13] and by pyridine derivatives[19] and triazines[20] bearing ω-aminoalkyl substituents. We report here on another category of ligand, based on the polyamide architecture that has been extensively explored for sequence-selective binding to duplex DNA. We have taken the tri-*N*-methyl-pyrrole duplex-DNA binding ligand distamycin A[21] (Fig. 1) as a lead molecule. Derivatives of distamycin A have been previously reported to be inhibitors of the human telomerase enzyme[22] although distamycin A itself lacks activity. Recent NMR studies[23,24] have suggested that distamycin A is also able to interact with G-quadruplex DNA. Two contrasting alternative models have been proposed in which (i) distamycin molecules bind as dimers in two of the four grooves of a quadruplex,[23] and (ii) in which two molecules of distamycin A extend over each of the two G-tetrad planes in a 4 : 1 binding mode.[24]

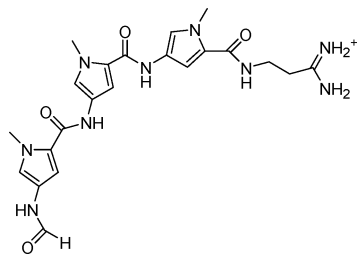

Fig. 1 Distamycin A.

We describe here the solid phase synthesis of a number of distamycin A polyamides and assess their ability to selectively bind to telomeric G-quadruplex DNA, in comparison with their binding to duplex DNA. Distamycin A is a classic DNA minor groove binder,[21] with selectivity for the narrow, deep minor groove of B-DNA A/T sequences, derived from its planar, curved isohelical structure.[25] The observation of the side-by-side binding of distamycin A in longer A/T sequences,[26] ultimately led to the design of the sequence-reading oligopyrrole carboxamide (polyamide) hairpin structures.[27] These molecules can be used to target particular DNA sequences and act as inhibitors of DNA–protein interactions.[28]

Key to the successful development of distamycin A polyamide molecules as quadruplex binding and stabilizing agents is the ability to selectively target G-quadruplex over duplex DNA. Using qualitative molecular modeling with the human intramolecular G-quadruplex structure,[29] we reasoned that if a 2,5-disubstituted pyrrole-carboxamide were also to adopt the isohelicity required to complement the duplex structure, it would then position the *N*-methyl group *into* rather than out of the groove, causing a steric clash that would inhibit binding to duplex DNA but would

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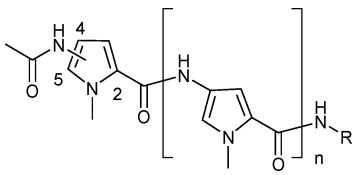
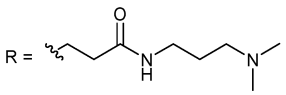
Table 1





n	2,4-Substituted oligomers	2,5-Substituted oligomers
1	**AcPy$_2$βAlaDp (1)**	**Ac(2,5-Py)PyβAlaDp (6)**
2	**AcPy$_3$βAlaDp (2)**	**Ac(2,5-Py)Py$_2$βAlaDp (7)**
3	**AcPy$_4$βAlaDp (3)**	**Ac(2,5-Py)Py$_3$βAlaDp (8)**
4	**AcPy$_5$βAlaDp (4)**	**Ac(2,5-Py)Py$_4$βAlaDp (9)**
5	**AcPy$_6$βAlaDp (5)**	**Ac(2,5-Py)Py$_5$βAlaDp (10)**

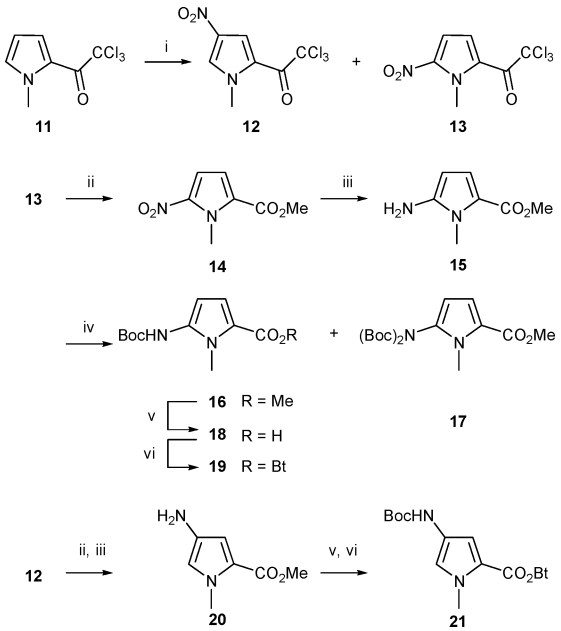
have little effect on G-quadruplex affinity. We initially assumed a structural model with distamycin bound on a terminal G-quartet of a quadruplex, analogous to the planar chromophore binding mode observed in quadruplex–ligand crystal structures,[30] and thus distamycin would not be intercalated between quartets.

Although our modeling studies suggested that steric crowding around a centrally-positioned 2,5-analogue may cause synthetic problems, we reasoned that the design of end-substituted (either amino or acid terminus) derivatives would be feasible and would allow the testing of the design. Loss of isohelicity also accompanies the synthesis of longer duplex-binding distamycin analogues,[31] but would not be expected to have an effect on a stacking binding mode to a G-quadruplex structure. Therefore, a series of distamycin A polyamides (**2–11**, Table 1) containing 2,5-disubstituted pyrroles and of increasing length were prepared by solid phase synthesis and their relative binding affinities for duplex DNA and G-quadruplex DNA determined by a FRET (fluorescence resonance energy transfer) assay modified by us[32] for this purpose.

Results

Monomer synthesis

Solid phase synthesis provides a rapid and effective route to extended polyamides.[33,34] For the synthesis to proceed, large amounts of the HOBt active ester of both the 2,4 and 2,5-regioisomers were required (Scheme 1). Synthesis of **19** and **21** followed modifications of the literature procedure reported for **21**.[33] A first point of consideration was a synthesis of **14** as a step towards **19**. The preparation of **14** has been achieved by nitration of commercially available 1-methylpyrrole-2-carboxylic acid followed by esterification to give a mixture which on separation affords **14** (11%) and the regioisomer methyl 4-nitro-1-methylpyrrole-2-carboxylate (42%).[35] A small amount of 2,4-dinitro-1-methylpyrrole may also be isolated.[36] The nitration of **11**, prepared from 1-methylpyrrole, has also been reported to proceed regioselectively with isolation of the 4-nitro-isomer in reasonable yield through fractional crystallization from isopropyl alcohol at −20 °C.[33] It had not been reported whether the nitrating



Scheme 1 Monomer synthesis. i) HNO_3 dropwise, Ac_2O, −40 °C–rt, $(CH_3)_2CHOH$, −40 °C ii) 0.05 equiv. DMAP, MeOH, rt, N_2 iii) H_2 (30 psi for 20), 10% Pd–C, THF (EtOAc for **20**) iv) 1.2 equiv. Boc_2O, 1.1 equiv. TEA, 1,4-dioxane, Δ v) 2 M aqueous NaOH, MeOH, 60 °C vi) HOBt, EDCI or DCC, DMF, rt, N_2.

mixture contained the 5-nitropyrrole or the 4,5-dinitropyrrole as described for the related nitration of 1-methylpyrrole-2-carboxylic acid. Investigation of this reaction indicated that the 5-nitro isomer **13** was present in the nitration mixture. As this route is amenable to very large scale synthesis[33] from cheap starting materials, we reasoned that the isolation of **13** from the nitration mixture could give significant amounts of material, albeit with a low but acceptable yield (18%) at this early stage. Hence this route was adopted towards a synthesis of both **12** and **13** as steps towards **21** and **19** respectively.

Trichloroacetylation proceeded as previously reported.[33] Nitration under the usual conditions was followed by fractional crystallization to isolate pure 4-nitro-regioisomer **12**. Chromatography of the crystallization solvent gave pure **13** in 18% yield, along with further amounts of **12**. Esterification of **13** (step ii, Scheme 1) was rapidly effected with catalytic DMAP in methanol at room temperature in 80% yield. Conditions utilizing sodium methoxide, as previously described,[33] were not required and, in our hands, this reaction also proceeds efficiently with the 2,4-regioisomer **12**. The difference in reactivity and solubility between the 2,5-disubstituted intermediates and their 2,4-counterparts manifested itself in several of the subsequent synthetic steps. Hydrogenation of **14** to **15** was achieved under milder conditions (4.5 h at ambient pressure and temperature) than those typically observed with the 2,4-disubstituted counterpart, where reaction required 30 psi of hydrogen and remained, at times, irreproducibly stubborn to even these conditions. The pyrrole amine **15**, obtained as a red residue, was immediately protected to give a mixture of mono- and di-Boc products **16** (isolated yield 57%) and **17** (isolated yield 2%). In the case of 2,4-disubstituted substrate **20** under identical conditions only the mono-protected product was observed. Hydrolysis of **16** by heating with a 1 : 1 methanol–water solution of sodium

hydroxide (7 equiv.) afforded **18** (79%). DCC or EDCI mediated esterification of the acid **18** gave **19** in 94% yield (6% overall yield from **11**).

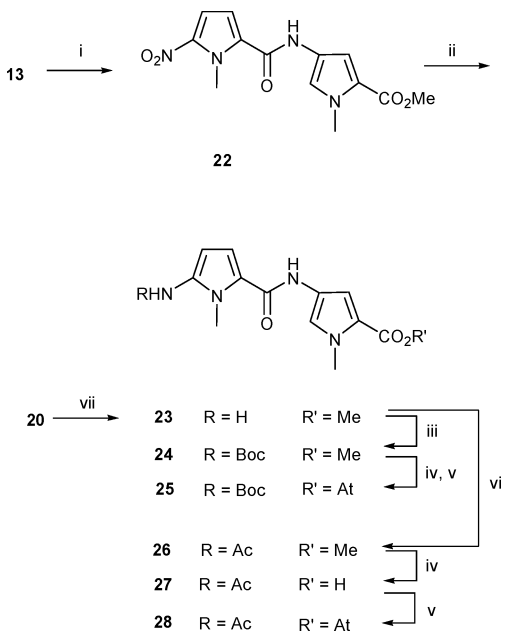
Solid phase synthesis

Manual solid phase synthesis of polyamides from **19** and **21** was undertaken using standard coupling conditions.[33,34] Chloranil has been described as an effective colorimetric test for the presence of aromatic amines[37] and in this synthesis we utilized a chloranil solution to assess coupling of the pyrrole amines. The syntheses of 2,4-pyrrole oligomers **1–5** proceeded as planned with two notable exceptions. Coupling between **21** and resin bound Py_5 amine required an extended overnight reaction time period beyond the standard 45 minutes. Acetylation of the resin bound Py_6 amine also required treatment beyond the standard 30 minute period. Repeated treatments with a mixture of Ac_2O, DIPEA in DMF of 1 h, 1.5 h and 2 h proceed with little conversion as indicated by the chloranil test. However, utilization of a freshly prepared mixture containing acetyl chloride (12 equiv.) and DIPEA (12 equiv.) in DMF proved successful in reducing the reaction times to 30 minutes in subsequent experiments.

The synthesis of the 2,5-pyrrole oligomer series **6–10** proved more demanding. To date, solid phase synthesis of polyamides possessing a centrally positioned 2,5-pyrrole has not been disclosed. The synthesis of trimer **7** was attempted first and achieved directly on the solid phase. Coupling of **19** to the resin bound Py_2 amine required repeated couplings and longer reaction times consisting of three cycles of 45, 90 and 90 minutes duration. However resin-bound Boc(2,5-Py)Py_2 deprotection and acetylation proceeded smoothly. Resin cleavage by treatment with 3-dimethylaminopropylamine gave **7** in a reasonable 55% yield following purification by amine scavenge.

Attempts at the introduction of the 2,5-substituted pyrrole at the *N*-terminus of longer polyamide chains ($n > 2$) proved fruitless. LC/MS analysis of cleavage products indicated not only failed acetylation but also failed couplings between the resin bound pyrrole amines and **19** raising concerns over both the efficiencies of this synthetic step and the sensitivity of chloranil as an analytical tool. An alternative approach to step-wise solid phase synthesis of polyamides has involved the coupling of a 'preformed' solution phase prepared dimer into the growing polyamide chain.[33,38] In this way the troublesome coupling of **19** to a resin pyrrole amine could be by-passed. We also sought to utilize the observed increased coupling efficiency of HOAt active esters and hence **26** was prepared.[34] Synthesis of **25** (Scheme 2) was initially achieved by the DMAP mediated coupling of **19** to **20** following Boger's related procedure[39] to give **24** (53%) followed by hydrolysis and esterification. However the expense incurred by the low yielding synthesis of **13** towards **19** and the expense of the latter's use in solid phase syntheses prompted the use of haloform chemistry[40] to direct a cheaper preparation of **24** and then **26**.

Reaction of **13** and amine **20** catalysed by DMAP gave **22** (81%) which on successive hydrogenation and Boc protection afforded **24** (44% over two steps). Hydrolysis and esterification gave **25** (8% overall yield from **13**). The use of dimer **25**, rather than monomer **19**, in coupling with resin bound pyrrole amines, proceeded with greater ease. However the subsequent deprotection acetylation cycle proved problematic. A bead colour change to purple on



Scheme 2 Dimer active ester synthesis. i) **20**, DMAP (0.2 equiv.), THF, N_2, rt, 2 h, 81% ii) 30 psi H_2, 10% Pd–C, EtOH, rt, 3 h iii) Boc_2O, TEA, 1,4-dioxane, 85 °C, 16 h, 44% for 2 steps iv) NaOH, H_2O–MeOH (1 : 1), 39% (**27**) v) EDCI, HOAt, DMF, rt, N_2 21% (**25** over 2 steps), 66% (**28**) vi) Ac_2O, DMAP, CH_2Cl_2, N_2, 38% vii) **19**, EDCI, DMAP, DMF, rt, N_2, 53%.

the required extended acetylation limited the use of chloranil but definitive LC/MS analysis of cleavage products indicated a lack of success. Rather than carrying out the final acetylation on bead we sought to undertake this in solution and hence the dimer Ac(2,5-Py)PyOAt (**28**) was adopted as a synthetic target. This was prepared by acetylation of **23** followed by hydrolysis and re-esterification. The subsequent success in coupling **28** to the appropriate pyrrole amine obviated the need to carry out the troubling solid phase acetylation and brought with it ultimately the synthesis of the remaining oligomers **6** and **8–10**.

Quadruplex-binding assay

Polyamides were examined in a FRET (fluorescence resonance energy transfer) assay to assess both their G-quadruplex DNA and duplex DNA stabilising abilities. The assay was conducted using the previously reported protocol.[32] In brief, changes in the FRET signal upon melting of telomeric G-quadruplex DNA and a duplex DNA probe were observed under conditions approximating those used in the crystallisation of the human intramolecular G-quadruplex DNA structure[29] (50 mM potassium cacodylate buffer, pH 7.4). Analysis of this data provides changes in melting point (ΔT_m), a measure of DNA stabilization and ligand binding affinity.

G-Quadruplex DNA stabilization

As polyamide length increases, an increase in G-quadruplex (G4) ΔT_m was observed within both series of ligands **1–5** and **6–10**. This increased G4 stabilization is not linear, being small for the tetramer **3** and even more so for compound **8**. Comparing G4 stabilization across the series it is apparent that the 2,5-pyrrole

dimer and trimer, compounds **6** and **7**, possess greater G4 DNA binding affinity than the corresponding 2,4-pyrrole oligomers **1** and **2**. This trend however is reversed when longer polyamides are considered. The 2,4-polyamides **4** and **5** are more potent than their 2,5-pyrrole counterparts **9** and **10**.

Duplex DNA stabilization

A near-perfect linear increase of ΔT_m with polyamide length was observed within the 2,4-pyrrole series **1–5**. The binding of compounds **6–10** to duplex DNA is not so consistent. Thus tetramer **8** has a high ΔT_m (16 °C) relative to the trimer **7** (1.3 °C) and pentamer **9** (8.5 °C). Across the two series, 2,5-pyrrole tetramer **8** has increased duplex DNA binding affinity relative to the 2,4-pyrrole tetramer **3**. This relationship exists for the dimers **6** and **1** but the magnitude of the difference is small. For the remaining members, substitution of a 2,4-pyrrole heterocycle (Py) with a 2,5-pyrrole heterocycle (2,5-Py) results in decreased duplex DNA binding affinity. Distamycin A, as control, has a ΔT_m of 20.5 °C, much higher than the stabilization produced by the synthetic trimers **2** (3.8 °C) and **7** (1.3 °C).

Discussion

We expected that polyamides beyond five contiguous rings would display reduced duplex DNA affinity,[16] as a consequence of the problem of helical phasing. The results of this study indicate that a plateau is yet to be reached after which introduction of additional pyrrole carboxamides for compounds **1–5** is penalized and duplex DNA stabilization decreases, at least for the experimental conditions employed here (Table 2). The ΔT_m values show that duplex DNA stabilization increases markedly after addition of a pyrrole carboxamide to the pentamers **4** (ΔT_m = 16.3 °C) and **9** (8.5 °C) to give hexamers **5** (25.3 °C) and **10** (22 °C) respectively. In addition the viability of the concept of elongating polyamides to enhance G-quadruplex binding at the expense of duplex DNA affinity is demonstrated by the present FRET results. Compounds **4**, **5** and **9** induce greater G4 stabilization than distamycin itself, and thus show enhanced relative quadruplex affinity.

G-Quadruplex *versus* duplex DNA stabilization

All compounds (except **5**) have decreased G4–duplex stabilization compared to distamycin itself. G4 DNA stabilization for the 2,4-pyrrole oligomers **1–5** follows the trend set for duplex DNA stabilization, and all display 2–4 fold selectivity for duplex DNA over G4 DNA. All the 2,5-pyrrole oligomers **6–10** display selectivity for duplex DNA, with the one notable exception being the trimer **7** which displays a preference for G4 DNA. This preference is qualified by the modest ΔT_m vales for both G4 DNA (3.3 °C) and duplex DNA (1.3 °C) although the ratio of melting temperatures (duplex DNA ΔT_m–G-Quadruplex DNA ΔT_m) is 0.4 whereas for the other polyamides reported here this ratio is always >1. The selectivity shown by **7** is a consequence more of its low duplex DNA stabilization than significant G-quadruplex stabilization. Polyamides **8** and **9** have comparable G4 DNA stabilization to **7** but also have significantly increased duplex DNA affinity. Here the addition of a pyrrole carboxamide seems advantageous for duplex binding but not for G-quadruplex binding. There is an increase in G4 stabilization observed for **9** relative to **7**, however this is accompanied by increased duplex stabilization. Compound **5** is notable in showing a high level of binding to both duplex and quadruplex DNA, so that the ratio of melting temperatures favours quadruplex some 3-fold more than distamycin itself.

The concept that incorporation of 2,5-pyrroles will promote selective G-quadruplex binding is borne out in principle by these FRET results. Comparison of melting temperature ratios (G-quadruplex DNA ΔT_m–duplex DNA ΔT_m) for dimers **1** (0.3) and **6** (0.55) indicates that incorporation of the 2,5-pyrrole into the polyamide scaffold has resulted in modest selectivity towards G4 DNA by decreasing duplex DNA binding affinity. However this favourable effect is diluted with considering longer sequences. 2,4-Pyrrole polyamides **4** and **5** have similar selectivity for G4 DNA over duplex DNA compared to their 2,5-pyrrole counterparts **9** and **10**. The unexpected finding here that the longer length polyamides show the greatest quadruplex stabilization, suggests that these may be binding in the grooves of a low-energy form of the human intramolecular quadruplex structure. However the short length of groove in these structures appears to be insufficient

Table 2 G-Quadruplex DNA and duplex DNA stabilization (FRET) for compounds **1–10**, distamycin and the established quadruplex-binding molecule, BRACO-19[14]

Compound	Duplex DNA[c]/$[\Delta T_m]_D$	G-Quadruplex DNA[b]/$[\Delta T_m]_Q$	Ratio $[\Delta T_m]_D$–$[\Delta T_m]_Q$
1	1	0.3	3.33
2	3.8	1.8	4.75
3	10.8	2.7	4.00
4	16.3	7.2	2.26
5	25.3	14	1.81
6	2	1.1	1.81
7	1.3	3.3	0.39
8	16	2.4	6.67
9	8.5	3.8	2.24
10	22	9.1	2.42
BRACO-19[14]	11	31	
Distamycin A	20.5	3.5	5.86

[a] Values reported are the average of two determinations at a compound concentration of 10 μM. [b] Using the labeled G-quadruplex telomeric sequence (5′-FAM-d[GGG(TTAGGG)$_3$]-TAMRA-3′). [c] Duplex DNA sequence d[TATATATATA] linked by hexaethyleneglycol and labeled with FAM and TAMRA at 5′ and 3′ ends respectively.

to accommodate the five or even six pyrrole-amide units in compounds **4**, **5** or **9**. This apparent inconsistency may be resolved by a binding model in which one end of these conformationally flexible polyamides stacks onto G-quartet ends, and the other is bound in a quadruplex groove. Such a model is stereochemically feasible (Fig. 2) although as yet there is no experimental evidence to support it.

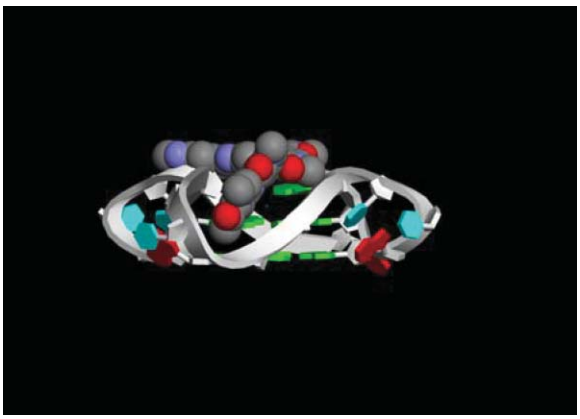


Fig. 2 A qualitative molecular model of a six-repeat pyrrole polyamide molecule (shown in space-filling mode), analogous to compound **5**, docked onto the crystal structure of the human 22-mer quadruplex structure[29] (with the bases and backbone shown in cartoon form). Two repeats of the polyamide are located in a quadruplex groove, and the others are stacked on a terminal G-quartet surface.

Inhibition of telomerase by distamycin A and compound **7** was determined by the modified PCR based TRAP assay which was performed as previously described.[32,41] The two compounds were examined in this way to a concentration of 100 μM and concomitant controls were carried out to verify that there was no interference with the correct functioning of *Taq* polymerase at these concentrations. Distamycin exhibited a $^{tel}EC_{50}$ of 25 μM whereas compound **7** showed no telomerase inhibitory activity at 100 μM. However, both compounds showed significant PCR inhibition, suggesting that the positive results in the TRAP assay were not necessarily indicative of telomerase inhibition *per se*.

Experimental

General

Microwave irradiation was performed with an Emrys Optimizer from Personal Chemistry. Melting points (mp) were measured with a Stuart Scientific SMP1 melting point apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer SPECTRUM 1000 FT-IR Spectrometer. Proton NMR spectra were acquired in $CDCl_3$, (Sigma-Aldrich) CD_3OD (GOSS), DMSO-d_6 (GOSS) solutions with chemical shift (δ) reported in ppm relative to the internal standard TMS or for CD_3OD solutions relative to the residual solvent resonance ($\delta = 3.31$). Carbon NMR spectra were recorded in $CDCl_3$ solutions referenced to the solvent peak ($\delta = 77.0$ ppm). LC/MS was performed using a Waters system combining a 2695 separation module, a Micromass ZQ spectrometer and a 2996 photodiode array detector. Mass spectrometry and elemental analysis services (ESI, HRMS) were provided by the School of Pharmacy. TLC analysis was carried out on silica gel (Merck 60F-254) with visualization at 254 and 366 nm and flash chromatography carried out with BDH silica gel (BDH 153325P). Treatment of an organic solution in the usual manner refers to stepwise drying with magnesium sulfate, filtration and then evaporation of the filtrate *in vacuo.* Reagents and chemicals (including anhydrous 1,4-dioxane), excepting HBTU and HOBT (Novabiochem), were purchased from Sigma-Aldrich. Solvents were purchased from BDH. Anhydrous THF was distilled from the ketal formed from sodium and benzophenone. The polyamide monomer **21** was prepared *via* the intermediates **11**, **12** and **20** according to the literature procedure[33] as was acid **29**.[42]

Polyamide synthesis

Solid phase synthesis of polyamides employed manual methodologies.[33,34] Thus this resin (0.75 mmol g^{-1}) was placed in a peptide reaction vessel and covered in DMF to swell for 1 h. The reaction vessel was drained and the resin washed (DCM (2 × 30 s) and DMF (1 × 30 s)). The Boc group was removed with 92.5 : 5 : 2.5 TFA–phenol–water (TPW) (1 × 30 s, 1 × 20 min) and the resin washed. Coupling consisted of the addition of the appropriate active ester (4 equiv.) in DMF, neat DIPEA (12 equiv.) and then shaking for the stated time. Colorimetric analysis of deprotection and coupling cycles was undertaken. Ninhydrin was used in the first coupling cycle and then subsequently a 2% DMF solution of chloranil (Avocado) was used as an indicator.[22] With a pyrrole amine on bead a resin sample was observed to turn brown to black otherwise a resin sample was colourless. Splitting was achieved by additional washing of the resin (2 × DCM) after a completed coupling cycle and drying under suction. The resin was weighed and divided by mass according to the desired splitting factor. Solid phase acetylation was carried out by shaking with freshly prepared mixture A for thirty minutes. The preparation of larger, especially mixed, polyamides required repeated treatments with mixture A hence in these cases this was substituted for mixture B. Mixture A contained acetic anhydride (12 equiv.) and DIPEA (12 equiv.) in DMF whilst mixture B contained DMAP (2.5 equiv.) as an extra additive. Cleavage was undertaken by heating resin with *N*,*N*-dimethylaminopropylamine (DAP) at 60 °C overnight. Product polyamides were isolated as oily solids according to Chamberlin and Krutzik.[34] Excess cleaving reagent DAP was removed from polyamide products by use of a nucleophile scavenging anhydride resin (Novabiochem MP anhydride), followed by purification by ion exchange chromatography (Isolute SCX2, Biotage). In this manner, polyamides **1–4** were constructed using a split synthesis from PAMβAlaBoc resin. Polyamide **2** has been reported previously.[43] The polyamide $AcPy_6\beta AlaDp$ (**5**) was prepared independently. Polyamide **7** was prepared by the coupling of **19** to the resin Py_2 amine followed by acetylation. Following inefficiencies with this protocol, polyamides **6**, **8–10** were prepared by coupling **28** to the respective resin pyrrole amine. The columns used were 500 mg SCX-2 columns. Samples were loaded into the column, dissolved in 2 ml of methanol. Non-basic compounds were washed off using methanol (2 × 2 ml) and 0.1 M ammonia in methanol (1 × 2 ml). The basic product was collected using 1 M ammonia in methanol (1 × 2 ml). The methanolic ammonia was evaporated off to yield the free base.

Biophysical studies

All oligonucleotides and their fluorescent conjugates were purchased from Eurogentec (Southampton, UK). DNA was initially dissolved as a stock 50 μM solution in purified water; further dilutions were carried out in the relevant buffer.

The ability of the compounds to stabilise G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used as a high-throughput screen in a 96-well format. The labelled oligonucleotide F21T (5′-*FAM*-dGGG(TTAGGG)$_3$-*TAMRA*-3′; donor fluorophore *FAM*: 6-carboxyfluorescein; acceptor fluorophore *TAMRA*: 6-carboxytetramethylrhodamine) used as the FRET probe was diluted from stock to the correct concentration (400 nM) in a 50 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 85 °C for 10 min, followed by cooling to room temperature in the heating block.

Compounds were stored as 10 mM stock solutions in DMSO; final solutions (at 2 × concentration) were prepared using 10 mM HCl in the initial 1 : 10 dilution, after which 50 mM potassium cacodylate buffer (pH 7.4) was used in all subsequent steps. The maximum HCl concentration in the reaction volume (at a ligand concentration of 20 μM) is thus 200 μM, well within the range of the buffer used. Relevant controls were also performed to ascertain a lack of interference with the assay. 96-Well plates (MJ Research, Waltham, MA) were prepared by aliquoting 50 μl of the annealed DNA into each well, followed by 50 μl of the compound solutions. Measurements were made on a DNA Engine Opticon (MJ Research) with excitation at 450–495 nm and detection at 515–545 nm. Fluorescence readings were taken at intervals of 0.5 °C over the range 30–100 °C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value. Final analysis of the data was carried out using a script written in the program Origin 7.0 (OriginLab Corp., Northampton, MA). The advanced curve-fitting function in Origin 7.0 was used for calculation of ΔT_m values.

Synthetic chemistry

5-Nitro-2-(trichloroacetyl)-1-methylpyrrole (13). A solution of **11** (10.10 g, 0.045 mol) in Ac_2O (60 ml) was treated dropwise with nitric acid (69%, 8 ml) over a period of 0.5 h at −40 °C in a CCl_4–dry ice bath. The reaction solution was allowed to warm to rt and then stirred for 3 h. The solution was then cooled to −40 °C, isopropyl alcohol (53 ml) added and the temperature maintained at −40 °C for 0.5 h and −22 °C overnight. The resultant brown solution was concentrated, poured into H_2O and the resulting mixture was extracted with $CHCl_3$. The organic extract was worked up in the usual manner to give a brown residue which solidified on standing. The solid was triturated with ice-cold EtOAc upon which a white solid (**12**), deposited. The solid was collected by filtration and washed with ice-cold EtOAc. The combined filtrate and washings were concentrated and stored at −22 °C overnight to provide a second crop of **12**. The brown residue resulting from evaporation of the filtrate was submitted to flash column chromatography (1 : 4 EtOAc–hexane) to afford, first, **13** as a pale green solid and then a further amount of **12**. Yield **13** (1.093 g, 18%): mp 67–69 °C; IR (cm^{-1}) 1692, 1540, 1503, 1353, 1290, 1210, 1183, 1130;^{1}H NMR ($CDCl_3$) δ ppm 4.28 (s, 3H), 7.15 (d, 2H, J = 4.7 Hz), 7.44 (d, 2H, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) δ 36.3, 95.2, 111.3, 120.4, 125.2, 142.9, 174.1.

Methyl 5-nitro-1-methylpyrrole-2-carboxylic acid ester (14)[35]. DMAP (38 mg, 0.3 mmol, 0.05 equiv.) was added portionwise to a stirred suspension of **13** (1.567 g, 5.8 mmol) in anhydrous CH_3OH (20 ml) under N_2. The suspension was warmed slightly to aid dissolution. After 15 min, a pale yellow solid precipitated and the resulting suspension was stirred for a further 1.25 h. The solid (**14**) was collected by filtration and a second crop isolated. Total yield **14** (852 mg, 80%): mp 104–106 °C (MeOH) (lit.[20] mp 114 °C); IR (cm^{-1}) 3121, 2363, 1710, 1532, 1495, 1476, 1303, 1247; ^{1}H NMR ($CDCl_3$) δ ppm 3.90 (s, 3H), 4.32 (s, 3H), 6.90 (d, 2H, J = 4.6 Hz), 7.13 (d, 2H, J = 4.5 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 35.2, 52.1, 112.0, 115.7, 126.9, 141.3, 160.6. Found C, 45.89; H, 4.30; N, 15.10. Calcd C, 45.66; H, 4.38; N, 15.21%.

Methyl 5-amino-1-methylpyrrole-2-carboxylate (15). Methyl ester **14** (146 mg, 0.8 mmol) in anhydrous THF was stirred under H_2 in the presence of 10% Pd–C (30 mg) for 4.5 h. The reaction mixture was filtered through Celite, the Celite was washed with EtOAc and the combined filtrate and washings were concentrated to give **15** as a red residue which was submitted directly to the next synthetic step. Yield (123 mg, quantitative).

Methyl 5-[(*tert*-butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate (16) and methyl 5-[(di-*tert*-butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate (17). A solution of the amine **15** (618 mg, 4 mmol) and Et_3N (0.6 mL, 446 mg, 4.4 mmol, 1.1 equiv.) in 1,4-dioxane was treated portionwise with Boc_2O (1.050 g, 4.8 mmol, 1.2 equiv.). The solution was then heated under reflux and reaction progress monitored by TLC. On completion, the solution was concentrated to give a mixture of **16** and **17**. Purification by flash chromatography (1 : 4 EtOAc–hexane) gave **17** (30 mg, 2%) and **16** (577 mg, 57%): **16** mp 87–88 °C; IR (cm^{-1}) 3300, 2978, 1697, 1561, 1486, 1239, 1154, 1105, 747; ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 9H), 3.76 (s, 3H), 3.79 (s, 3H), 6.02 (d, 1H, J = 3.6 Hz), 6.50 (bs, 1H), 6.89 (d, 1H, J = 4.2 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 161.6, 153.5, 132.2, 119.2, 116.7, 103.0, 81.3, 50.9, 31.4, 28.1; HRMS (+ESI) m/z 255.1351 (MH^+, $C_{12}H_{19}N_2O_4$ requires 255.1339). **17** Mp 110–112 °C; IR (cm^{-1}): 2978, 1759, 1732, 1702, 1367, 1249, 1105; ^{1}H NMR ($CDCl_3$) δ ppm 1.35 (s, 18H), 3.63 (s, 3H), 3.74 (s, 3H), 5.91 (d, 1H, J = 4.2 Hz), 6.83 (d, 1H, J = 4.2 Hz); ^{13}C NMR ($CDCl_3$) δ 27.7, 31.1, 50.9, 83.3, 105.6, 116.2, 120.2, 132.3, 150.6, 161.5; HRMS (+ESI) m/z 355.1862 (MH^+, $C_{17}H_{27}N_2O_6$ requires 355.1864).

5-[(*tert*-Butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylic acid (18). A slurry of **6** (175 mg, 0.69 mmol) in a 1 : 1 solution of CH_3OH–2 M aq. NaOH (2.56 ml) was heated at 60 °C for 2 h. The resulting pink solution was allowed to cool to rt, washed with diethyl ether, acidified to pH = 1 (universal indicator paper) with 10% aq. HCl and extracted with EtOAc. The combined organic extracts were treated in the usual manner to give a tan residue which was dissolved in CH_2Cl_2 and a volume of hexane (4 × volume of CH_2Cl_2 used) was added. The resulting slurry was concentrated. This was repeated three times to give **19** as an orange

solid (130 mg, 79%): mp 110–113 °C; IR (cm^{-1}) 3317, 2981, 1697, 1651, 1530, 1461, 1365, 1246; ^{1}H NMR (DMSO-d_6) δ ppm 1.44 (s, 9H), 3.63 (s, 3H), 5.90 (d, 1H, J = 4.1 Hz), 6.74 (d, 1H, J = 4.1 Hz), 8.99 (s, 1H), 11.97 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm 27.9, 31.2, 79.4, 102.2, 116.0, 118.8, 133.2, 153.5, 161.8.

1,2,3-Benzotriazolyl-5-[(*tert*-butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate (19). The acid **18** (130 mg, 0.5 mmol) was dissolved in DMF (2 ml) and HOBt (80 mg, 0.5 mmol, 1 equiv.) and DCC (156 mg, 0.76 mmol, 1.4 equiv.) were added. The reaction mixture was microwaved at 80 °C for 20 minutes in fixed time mode at which point TLC analysis indicated reaction completion. The reaction mixture was filtered, and then evaporated to give an oil which was dissolved in EtOAc and passed through a silica plug to give **19** as a brown solid (177 mg, 94%): mp 109–113 °C, IR (cm^{-1}) 2977, 2931, 1761, 1731, 1558, 1364, 1227, 1149, 1044; ^{1}H NMR ($CDCl_3$) δ ppm 1.49 (s, 9H), 3.76 (s, 3H), 6.29 (d, 1H, J = 4 Hz), 7.36–7.53 (m, 4H), 7.98 (s, 1H), 8.03 (d, 1H, J = 9 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 156.6, 152.8, 143.4, 137.3, 129.2, 128.5, 124.7, 121.4, 120.3, 112, 108.6, 103.5, 81.9, 31.7, 28.2; HRMS (+ESI) m/z 358.1515 (MH^+, $C_{17}H_{20}N_5O_4$ requires 358.1510).

Methyl 4-(5-nitro-1-methylpyrrole-2-carboxamido)-1-methylpyrrole-2-carboxylate (22). A solution of **13** (1.608 g, 5.9 mmol) in anhydrous THF (5 ml) was added to a stirred solution of **21** (902 mg, 5.9 mmol) in anhydrous THF (10 ml) at rt. DMAP (142 mg, 1.2 mmol, 0.2 equiv.) was added and stirring continued. After 2 h a yellow solid (**22**) had precipitated and analysis of the reaction mixture by LC/MS showed the reaction to be complete. Filtration gave a first crop (953 mg) and concentrating the filtrate and crystallisation of the resulting residue from chloroform–hexane afforded a second crop (507 mg). The total yield of **22** was 1.46 g (81%): mp 166 °C (dec.); IR (cm^{-1}) 3342, 1691, 1670, 1566, 1439, 1348, 1292, 1239; ^{1}H NMR (DMSO-d_6) δ ppm 3.75 (s, 3H), 3.86 (s, 3H), 4.15 (s, 3H), 6.88 (d, 1H, J = 4.6 Hz), 6.92 (d, 1H, J = 2.0 Hz), 7.28 (d, 1H, J = 4.6 Hz), 7.50 (d, 1H, J = 1.9 Hz), 10.53 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 35.4, 36.9, 51.2, 106.4, 108.2, 110.1, 112.4, 112.8, 120.4, 120.9, 121.2, 131.3, 161.3; HRMS (ESI+) m/z 307.1029 (MH^+, $C_{13}H_{15}N_4O_5$ requires 307.1037).

Methyl (5-amino-1-methylpyrrole-2-carboxamido)-1-methylpyrrole-2-carboxylate (23). A solution of **22** (20 mg, 0.39 mmol) in ethanol (20 ml) containing 10% Pd–C (15 mg) was shaken under 30 psi of H_2 for 3 h. At reaction completion the mixture was filtered through Celite and concentrated to give **23** as a red residue, which was submitted directly to the next synthetic step.

Methyl 4-{5-[(*tert*-butyloxycarbonyl)amino]-1-methylpyrrole-2-carboxamido}-1-methylpyrrole-2-carboxylate (24). Triethylamine (0.059 mL, 43 mg, 0.42 mmol, 1.1 equiv.) and Boc_2O (70 mg, 0.27 mmol, 0.7 equiv.), were added sequentially and portionwise to a solution of the amine **23** (0.39 mmol) in anhydrous 1,4-dioxane (1.7 ml). The resulting solution was heated at 85 °C overnight under N_2. The reaction solution was cooled and concentrated to give a red-brown residue. Purification by flash chromatography (3 : 1 EtOAc–hexane) gave **24** as a pale brown solid (65 mg, 44%): mp 138–139 °C; IR (cm^{-1}) 1701, 1636, 1552, 1446, 1242, 1155, 1107; ^{1}H NMR ($CDCl_3$) δ ppm 1.50 (s, 9H), 3.75 (s, 3H), 3.80 (s, 3H), 3.89 (s, 3H), 5.99 (m, 1H), 6.32 (bs, 1H), 6.56 (d, 1H, J = 4.1 Hz), 6.75 (d, 1H, J = 2.0 Hz), 7.40 (d, 1H, J = 1.9 Hz), 7.77 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 28.2, 31.4, 36.7, 51.1, 81.3, 103.2, 108.3, 110.7, 119.7, 121.0, 121.9, 122.8, 130.9, 153.9, 159.3, 161.6; HRMS (+ESI) m/z 377.1819 (MH^+, $C_{18}H_{25}N_4O_5$ requires 377.1826).

Compound **24** was also prepared from the reaction between **19** and **20** in 53% yield after purification by flash chromatography.

7-Azabenzo-1,2,3-triazolyl 4-[5-[(*tert*-butoxycarbonyl)amino-1-methylpyrrole-2-yl]-carbonyl]amino-1-methylpyrrole-2-carboxylate (25). A suspension of the methyl ester **24** (30 mg, 0.08 mmol) in aq. NaOH (0.8 M, 1 mL, 0.8 mmol, 10 equiv.) and CH_3OH (1 ml) was heated at 50–60 °C overnight. The resulting yellow solution was washed with diethyl ether and acidified with 10% HCl. Extraction with EtOAc and the usual work up gave a brown residue. This was dissolved in CH_2Cl_2 and hexane was added and the suspension concentrated to dryness. This procedure was repeated twice to give the corresponding acid as a pale yellow solid. A solution of this solid (112 mg, 0.31 mmol) in anhydrous DMF (1.5 ml) was treated with HOAt (42 mg, 0.31 mmol) and EDCI (60 mg, 0.31 mmol). The reaction mixture was stirred under a N_2 atmosphere for 4 h, filtered through Celite into ice–water (15 ml) and the resulting suspension was filtered to give a pale yellow solid. This solid was dissolved in acetone (3 ml) and added to cold hexane (15 ml). The isolated solid was redissolved in CH_2Cl_2 (3 ml) and this solution was added to cold hexanes (15 ml). The pale yellow solid was collected by filtration and dried *in vacuo*. Yield **25** (75 mg, 21% over two steps): mp 139–143 °C; IR (cm^{-1}) 2981, 2358, 1687, 1555, 1447, 1390, 1243, 1157; ^{1}H NMR ($CDCl_3$) δ ppm 1.50 (s, 9H), 3.83 (s, 3H), 3.91 (s, 3H), 6.06 (d, 1H, J = 3.5 Hz), 6.24 (bs, 1H), 6.64 (d, 1H, J = 4.2 Hz), 7.25 (d, 1H, J = 1.9 Hz), 7.45 (dd, 1H, J = 4.5, 8.4 Hz), 7.76 (d, 1H, J = 1.7 Hz), 8.45 (dd, 1H, J = 1.4, 8.4 Hz), 8.74 (dd, 1H, J = 1.4, 4.5 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 28.2, 31.5, 36.8, 81.4, 103.1, 111.0, 111.2, 113.3, 115.9, 120.8, 122.3, 123.4, 125.2, 129.5, 131.4, 135.0, 141.0, 151.7, 156.3, 159.2; HRMS (+ESI) m/z 481.1919 (MH^+, $C_{22}H_{25}N_8O_5$ requires 481.1942).

Methyl 4-(5-acetamido-1-methylpyrrole-2-carboxamido)-1-methylpyrrole-2-carboxylate (26). Acetic anhydride (175 μL, 190 mg, 1.9 mmol, 1.2 equiv.) and DMAP (484 mg, 4 mmol, 2.5 equiv.) were added to a solution of amine **23** (0.44 g, 1.6 mmol) in anhydrous CH_2Cl_2 (15 ml) at rt under N_2. Stirring was continued for 3 h at which point LC/MS analysis showed the reaction to be complete. The mixture was concentrated, loaded onto a silica cartridge and eluted with EtOAc to give **26** as a yellow oil (193 mg, 38%). IR (cm^{-1}) 2359, 1700, 1663, 1625, 1540, 1444, 1245; ^{1}H NMR (DMSO-d_6) δ ppm 2.04 (s, 3H), 3.65 (s, 3H), 3.73 (s, 3H), 3.83 (s, 3H), 5.95 (d, 1H, J = 4 Hz), 6.84 (d, 1H, J = 4.1 Hz), 6.87 (d, 1H, J = 1.9 Hz), 7.44 (d, 1H, J = 1.9 Hz), 9.68 (bs, 1H), 9.81 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ ppm 22.7, 31.5, 36.2, 50.9, 101.7, 108.2, 111.4, 118.5, 120.6, 122.1, 122.9, 131.7, 158.6, 160.7, 169.2; HRMS (+ESI) m/z 319.1409 (MH^+, $C_{15}H_{19}N_4O_4$ requires 319.1401).

4-(5-Acetamido-1-methylpyrrole-2-carboxamido)-1-methylpyrrole-2-carboxylic acid (27). The methyl ester **26** (293 mg, 0.92 mmol) was suspended in 1 : 1 NaOH (aq. 2 M)–CH_3OH (4.6 ml) and heated at 45 °C for 2 h. The resulting solution was

cooled and then washed with diethyl ether, acidified to pH = 1 (universal indicator paper) with 10% HCl and then extracted with EtOAc. The organic extracts were treated in the usual manner to give **27** as a pale brown solid which was dried *in vacuo.* Yield (108 mg, 39%): mp 213–215 °C; IR (cm^{-1}) 3253, 1666, 1626, 1537, 1448, 1250; ^{1}H NMR (acetone-d_6) δ ppm 1.95 (s, 3H), 3.64 (s, 3H), 3.75 (s, 3H), 5.86 (d, 1H, J = 4 Hz), 6.72 (s, 1H), 6.77 (s, 1H), 7.31 (s, 1H), 9.20 (bs, 1H), 9.34 (bs, 1H), 11.65 (bs, 1H); ^{13}C NMR (acetone-d_6) δ ppm 24.0, 33.0, 37.6, 103.3, 110.1, 112.9, 121.7, 121.8, 121.9, 125.1, 133.9, 160.7, 164.4, 170.6; HRMS (+ESI) m/z 305.1220 (MH^+, $C_{14}H_{17}N_4O_4$ requires 305.1244).

7-Azabenzo-1,2,3-triazolyl 4-(5-acetamido-1-methylpyrrole-2-carboxamido)-1-methylpyrrole-2-carboxylate (28). A solution of the acid **27** (80 mg, 0.25 mmol) in DMF (3 ml) was treated successively with HOAt (34 mg, 0.25 mmol, 1 equiv.) and EDCI (48 mg, 0.25 mmol, 1 equiv.) and the resulting mixture was stirred at rt overnight under N_2. The red suspension was filtered into a stirred ice–water mixture (15 ml) at which point a pale yellow solid precipitated. The ice–water mixture was allowed to melt and was then filtered and the isolated solid partially dissolved in a small volume of CH_2Cl_2 and added to ice-cold hexanes (15 ml). The resulting suspension was filtered to give **28** as a pale yellow solid which was dried under suction and then *in vacuo.* Yield **28** (70 mg, 66%): mp 159–162 °C; IR (cm^{-1}) 3322, 2361, 1780, 1666, 1555, 1392, 957; ^{1}H NMR ($CDCl_3$) δ ppm 8.74 (dd, 1H, J = 1.1, 4.5 Hz), 8.45 (dd, 1H, J = 1.1, 8.4 Hz), 7.83 (s, 1H), 7.76 (d, 1H, J = 1.3 Hz), 7.45 (dd, 1H, J = 4.5, 8.3 Hz), 7.12 (s, 1H), 6.65 (d, 1H, J = 4.1 Hz), 6.09 (d, 1H, J = 4.1 Hz), 3.91 (s, 3H), 3.80 (s, 3H), 2.22 (s, 3H); ^{13}CNMR ($CDCl_3$) δ ppm 23.1, 31.7, 36.7, 103.2, 111.3, 111.7, 112.9, 120.7, 122.6, 123.9, 125.2, 126.0, 129.4, 131.2, 134.9, 140.9, 151.6, 156.3, 170.1; HRMS (+ESI) m/z 423.1543 (MH^+, $C_{19}H_{19}N_8O_4$ requires 423.1524)

AcPy$_2$βAlaDp (1)[41]. Resin PAMβAlaPy$_2$Boc (0.032 mmol) was deprotected and acetylated. Polyamide isolation followed by purification by ion-exchange chromatography afforded **1** (6 mg, 43%). ^{1}H NMR (CD_3OD) δ ppm 1.66–1.72 (m, 2H), 2.07 (s, 3H), 2.20 (s, 6H), 2.36 (m, 2H), 2.44–2.49 (m, 2H), 3.19–3.26 (m, 2H), 3.55 (t, 2H, J = 6.7 Hz), 3.87 (s, 3H), 3.89 (s, 3H), 7.18 (d, 1H, J = 1.6 Hz), 7.13 (d, 1H, J = 1.6 Hz), 6.81 (d, 1H, J = 1.6 Hz), 6.76 (d, 1H, J = 1.7 Hz); HRMS (+ESI) m/z 460.2663 (MH^+, $C_{22}H_{34}N_7O_4$ requires 460.2667).

AcPy$_4$βAlaDp (3). Resin PAMβAlaPy$_3$Boc (0.033 mmol) was deprotected and coupled to **20** (4 equiv.) with addition of DIPEA (12 equiv.) and shaking for 45 minutes. Deprotection and acetylation was followed by cleavage, polyamide isolation and purification by ion-exchange chromatography to give **3** (5 mg, 22%). ^{1}H NMR (CD_3OD) δ ppm 1.69 (m, 2H), 1.92 (s, 3H), 2.07 (s, 6H), 2.31–2.42 (m, 2H), 2.47 (t, 2H, J = 6.5 Hz), 3.21 (m, 2H), 3.55 (t, 2H, J = 6.7 Hz), 3.87–3.91 (m, 12H), 6.79–6.86 (m, 4H), 7.15–7.22 (m, 4H); HRMS (+ESI) m/z 704.3647 (MH^+, $C_{34}H_{46}N_{11}O_6$ requires 704.3627).

AcPy$_5$βAlaDp (4). Resin PAMβAlaPy$_4$Boc (0.035 mmol) was deprotected and coupled to **20** (4 equiv.) with addition of DIPEA (12 equiv.) and shaking for 45 minutes. Deprotection and acetylation was followed by polyamide isolation and purification by ion-exchange chromatography to afford **4** (14 mg, 48%). ^{1}H NMR (CD_3OD) δ ppm 1.83 (m, 2H), 1.92 (s, 3H), 2.05 (s, 6H), 2.50 (t, 2H, J = 6.4 Hz), 2.86–2.90 (m, 2H), 3.27 (m, 2H), 3.58 (t, 2H, J = 5.9 Hz), 3.87–3.91 (m, 15H), 6.84 (m, 2H), 6.96 (m, 2H), 7.13 (d, 1H, J = 1.2 Hz), 7.17 (d, 1H, J = 1.2 Hz), 7.21–7.20 (m, 4H); HRMS (+ESI) m/z 826.4091 (MH^+, $C_{40}H_{52}N_{13}O_7$ requires 826.4107).

AcPy$_6$βAlaDp (5). PAMβAlaPy$_6$Boc (0.52 mmol) was prepared from PAMβAlaBoc resin employing stepwise coupling of **29** (twice), activated by stirring with HBTU and HOBt in DMF prior to addition to resin, and then **21** (twice). Deprotection and acetylation using mixture B (4 × 1 h treatments) followed. Polyamide isolation gave an oily solid of which a sample was purified by silica column chromatography eluting with 3 : 6 : 1 CH_3OH–CH_2Cl_2–Et_3N to afford **5** (3 mg, 43%). ^{1}H NMR (CD_3OD) δ ppm 1.69 (m, 2H), 1.92 (s, 3H), 2.07 (s, 6H), 2.34 (m, 2H), 2.48 (t, 2H, J = 6.5 Hz), 3.22 (m, 2H), 3.56 (t, 2H, J = 6.7 Hz), 3.88–3.93 (m, 18H), 6.78 (d, 1H, J = 1.8 Hz), 6.84 (d, 1H, J = 1.8 Hz), 6.94–6.95 (m, 4H), 7.13 (d, 1H, J = 1.7 Hz), 7.21–7.19 (m, 5H); HRMS (+ESI) m/z 948.4621 (MH^+, $C_{46}H_{58}N_{15}O_8$ requires 948.4588).

2,5-Pyrrole polyamides (AcPy$_{n=2\text{-}6}$βAlaDp, 6–10)

Ac(2,5-Py)PyβAlaDp (6). Resin PAMβAlaBoc (26 mg, 0.02 mmol) was deprotected and coupled to **28** (24 mg, 0.057 mmol, 3 equiv.) with addition of DIPEA (0.041 mL, 30 mg, 0.24 mmol, 12 equiv.) and shaking overnight. The resin was then capped by shaking with an acetylation mixture containing Ac_2O (12 equiv.) and DIPEA (12 equiv.) in DMF for 1 h. Polyamide isolation and then purification by ion-exchange chromatography gave **6** (3 mg, 33%). ^{1}H NMR (CD_3OD) δ ppm 1.76 (m, 2H), 1.92 (s, 3H), 2.16 (s, 6H), 2.60 (m, 2H), 3.33 (m, 2H), 3.42 (t, 2H, J = 6.8 Hz), 3.57 (t, 2H, J = 6.7 Hz), 3.73 (s, 3H), 3.87 (s, 3H), 6.01 (d, 1H, J = 4.2 Hz), 6.78 (d, 1H, J = 1.9 Hz), 6.81 (d, 1H, J = 4.1 Hz), 7.15 (d, 1H, J = 1.7 Hz); HRMS (+ESI) m/z 460.2675 (MH^+, $C_{22}H_{34}N_7O_4$ requires 460.2667).

Ac(2,5-Py)Py$_2$βAlaDp (7). Prepared from PAMβAlaPyBoc (29 mg, 0.019 mmol) by coupling to **20** (two cycles required of 45 minutes' duration) and then **19** (45 minutes). Acetylation, cleavage and polyamide isolation and purification gave **7** (6 mg, 55%). ^{1}H NMR (CD_3OD) δ ppm 1.71 (m, 2H), 1.92 (s, 3H), 2.21 (s, 6H), 2.46 (m, 2H), 2.62 (m, 2H), 3.56 (t, 2H, J = 6.8 Hz), 3.75 (s, 3H), 3.88 (s, 3H), 3.91 (s, 3H), 6.02 (d, 1H, J = 4.1 Hz), 6.77 (d, 1H, J = 1.9 Hz), 6.82 (d, 1H, J = 4.1 Hz), 6.92 (d, 1H, J = 1.9 Hz), 7.19 (m, 2H), 7.99 (bs, 1H); HRMS (+ESI) m/z 582.3148 (MH^+, $C_{28}H_{40}N_9O_5$ requires 582.3147).

Ac(2,5-Py)Py$_3$βAlaDp (8). Prepared from the resin PAMβAlaPy$_2$Boc (0.0133 mmol) by coupling to **29** for 90 minutes. Cleavage and polyamide isolation gave **8** (5 mg, 54%). ^{1}H NMR (CD_3OD) δ ppm 1.73 (m, 2H), 2.14 (s, 3H), 2.35 (s, 6H), 2.46–2.54 (m, 4H), 3.21–3.24 (m, 2H), 3.56 (t, 2H, J = 6.6 Hz), 3.73 (s, 3H), 3.86 (s, 3H), 3.90 (s, 3H), 3.90 (s, 3H), 6.01 (d, 1H, J = 4.1 Hz), 6.78 (d, 1H, J = 1.9 Hz), 6.82 (d, 1H, J = 4.1 Hz), 6.928–6.932 (m, 2H), 7.17–7.19 (m, 3H); HRMS m/z 704.3659 (MH^+, $C_{34}H_{46}N_{11}O_6$ requires 704.3627).

Ac(2,5-Py)Py$_4$βAlaDp (9). Prepared from resin PAMβAlaPy$_2$Boc (0.027 mmol) by coupling to **20**, a two-fold split and then coupling to **29** for 90 minutes. Cleavage and polyamide isolation gave **9** (4 mg, 18%). ^{1}H NMR (CD$_3$OD) δ ppm 1.77 (m, 2H), 2.16 (s, 3H), 2.47 (s, 6H), 2.49 (t, 2H, J = 6.4 Hz), 2.65 (m, 2H), 3.25 (t, 2H, J = 6.7 Hz), 3.57 (t, 2H, J = 6.6 Hz), 3.72 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 3.89 (s, 6H), 6.02 (d, 1H, J = 4.1 Hz), 6.81 (d, 1H, J = 1.8 Hz), 6.83 (d, 1H, J = 4.1 Hz), 6.95–6.96 (m, 3H), 7.18 (d, 1H, J = 1.8 Hz), 7.20 (d, 1H, J = 1.8 Hz), 7.21 (d, 2H, J = 1.8 Hz); HRMS m/z 826.4110 (MH$^+$, $C_{40}H_{52}N_{13}O_7$ requires 826.4107).

Ac(2,5-Py)Py$_5$βAlaDp (10). Prepared from resin PAMβAlaPy$_3$Boc (0.0133 mmol) by coupling to **20** and then **29** for 135 minutes. Cleavage and polyamide isolation gave **10** (5 mg, 40%). ^{1}H NMR (CD$_3$OD) δ ppm 1.66 (m, 2H), 2.14 (s, 3H), 2.19 (s, 6H), 2.31 (t, 2H, J = 7.9 Hz), 2.45 (t, 2H, J = 6.7 Hz), 2.71 (m, 2H), 3.53 (t, 2H., J = 6.5 Hz), 3.74 (s, 3H), 3.86 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 3.91 (s, 6H), 6.00 (d, 1H, J = 4.1 Hz), 6.75 (d, 1H, J = 1.7 Hz), 6.81 (d, 1H, J = 4.1 Hz), 6.92–6.93 (m, 4H), 7.18 (m, 5H); HRMS (+ESI) m/z 948.4636 (MH$^+$, $C_{46}H_{58}N_{15}O_8$ requires 948.4587).

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